

## Supplemental Information

### Reprogramming acyl carrier protein interactions of an extender unit promiscuous trans-acting acyltransferase

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#### Inventory of Supplemental Information

**Table S1:** Identity of ACP residues involved in protein interactions with various domains, related to Figure 7.

**Table S2:** Oligonucleotides used for cloning of kirromycin ACP's, related to 'Cloning and Expression of Kirromycin ACP's' and Figure 4.

**Table S3:** Oligonucleotides used for construction of ACP10<sub>Kir</sub>/ACP5<sub>Kir</sub> chimeras, related to 'Site-Directed Mutagenesis and Chimeragenesis of ACP's and KirCII' and Figure 7.

**Figure S1:** LC-MS analysis of the conversion of apo-ACP<sub>Kir</sub> to holo-ACP<sub>Kir</sub>. These spectra show the complete Sfp-catalyzed phosphopantetheinylation of wild-type apo-ACP5<sub>Kir</sub> as a representative example, related to 'Cloning and Expression of Kirromycin ACP's'.

**Figure S2:** CD spectra of wild-type and mutant ACP5<sub>Kir</sub>. These spectra show that the mutations analyzed do not significantly alter the secondary structure, compared to the wild-type ACP, related to 'Cloning and Expression of Kirromycin ACP's'.

**Figure S3:** Amino acid sequence alignment of the KirCII with DEBS AT's (AT1–AT6). This illustrates homology between the N-terminal portion of KirCII and the KS-AT linker domains of the DEBS AT's, related to Figure 6.

**Figure S4:** Structural homology between the KirCII KS-AT linker domain and the known AT3 linker of DEBS, related to Figure 6.

**Figure S5:** CD spectra of wild-type and mutant KirCII, related to Figure 6.

**Figure S6:** Secondary structure prediction of ACP5<sub>Kir</sub> by PSIPRED, related to Figure 7.

**Figure S7:** Amino acid alignment of various ACP's, related to Figure 7.

**Table S1:** Identity of ACP residues involved in protein interactions with various domains, related to Figure 7.

ACP5 <sub>Kir</sub> residue	Act-ACP: MCAT	ACP: DSZS	DYN10 ACP: KSAT	DEBS ACP2: KS3-AT3	DEBS ACP3: KS3-AT3	DEBS ACP4: KS4-AT4	CurAC P1: Hal	PKSA ACP: AT	MupA 3a: MupH	AcpP: FabH	AcpP: BioH	AcpP: P450	AcpP: SpoT	AcpP: FabI	AcpP: FabA	EntF PCP: TE	EntB ArCP: EntF	Sc FAS ACP: MCAT
	Ref a	Ref b	Ref c	Ref d	Ref e	Ref f	Ref g	Ref h	Ref i	Ref j	Ref k	Ref l	Ref m	Ref n	Ref o	Ref p	Ref q	Ref r
D28				E														
P29				R														
E30				T														
S31				A														
A32				E														
V33				L						R						L		
R34				V														
D35				R														
A42			A														G	
A43			E													V		
H44											Q	Q		Q				
L45																		
A48	D																	
R51																		
L52					E													
P53					I													
P54			R		N										N			
D55					R													
R56					A					R								
V57					S					L								
L58					F													
S59					S													
D60			D		E	D		D	E									
V61			L		L	L												
G62			H															
V63			L			F												
D64	D	D	N		D	D	D				D			D				D
S65						S			S	S					S			
L66						L	I		V		L							
G67			R							D		D		D	D			
R69									A	V	V				V		M	
R70				R								E		E	E			E
L71																		
S72					R								V					
R73									R	M	M							
R74				L						A					A			
G76										E	E				E			E
A77										E		E		E				E
T78										E								
Y79													F					
D82				R	R										E			
I83										I		I	I					
P84								E										
A85							A			D		D					F	
R86							T		D									
F88																		
G89					F		Y		Y					E	M	A		
V90					D	D	D		T								K	
Q92	D																	
T93													T					
A96		Q																
R97		R																
A100									W									
D103									T									
K104									Q									

Only those residues important for interactions are shown. Key ACP5<sub>Kir</sub> residues discussed in the text are color coded as follows: **Orange**, key electrostatic interactions from ACP5<sub>Kir</sub> alanine scanning and docking studies; **Red**, positions of mutations in the HII' region (discussed in text); **Green**, phosphopantetheinylation site. References: a) Keatinge-Clay et al., *Structure* **2003**, 11, 147; b) Wong et al., *Biochem* **2011**, 50, 6539; c) Liew et al., *J Biol Chem* **2012**, 287, 23203; d) Kapur et al., *PNAS* **2010**, 107, 22066; e) Tang et al., *Chem Biol* **2007**, 14, 931; f) Taylor et al., *J Biomol Struct Dynam* **2013**, 31, 17; g) Busche et al., *ACS Chem Biol* **2012**, 7, 378–86; h) Bruegger et al., *Chem Biol* **2013**, 20, 1135; i) Haines et al., *Nat Chem Biol* **2013**, 9, 685; j) Zhang et al., *J Biol Chem* **2011**, 276, 8231; k) Agarwal et al., *PNAS* **2012**, 109, 17406; l) Cryle et al., *PNAS* **2008**, 105, 15696; m) Angelini et al., *PLoS One* **2012**, 7, e36111; n) Rafi et al., *J Biol Chem* **2006**, 281, 39285; o) Worsham et al., *Biochemistry*, **2003**, 42, 167; p) Zhou et al., *Chem Biol* **2006**, 13, 869; q) Lai et al., *PNAS* **2006**, 103, 5314; r) Arthur et al., *ACS Chem. Biol.*, **2009**, 4, 625.

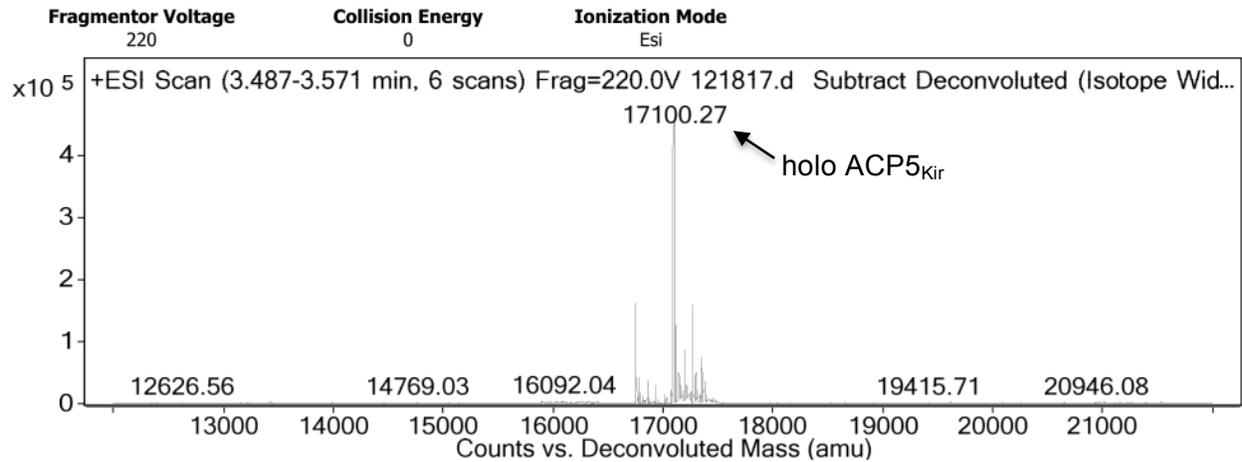
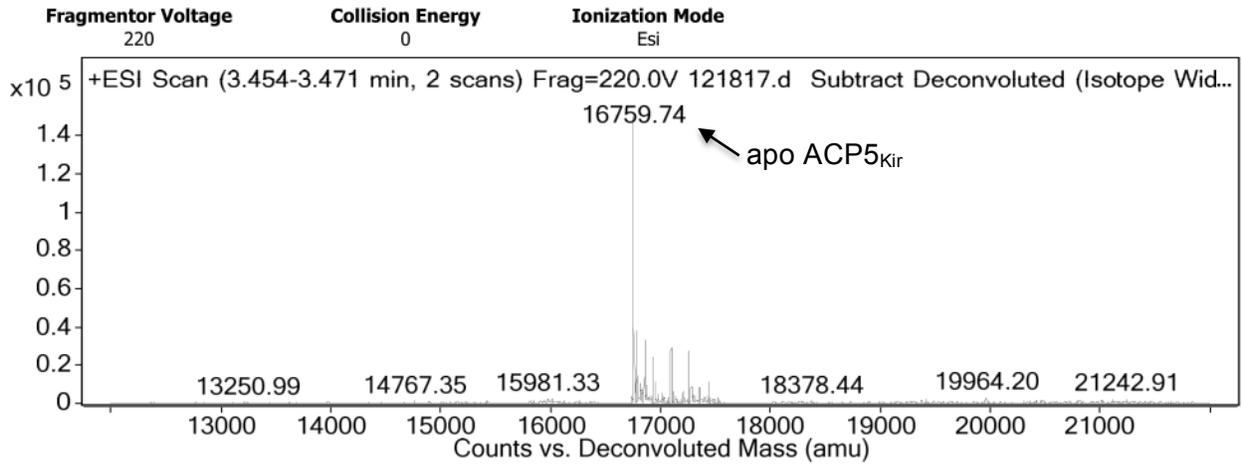
**Table S2:** Oligonucleotides used for cloning of kirromycin ACPs, related to 'Cloning and Expression of Kirromycin ACP's' and Figure 4.

<b>Primer name</b>	<b>Sequence</b>
5-ACP0pET30LIC	GACGACGACAAGATGTGGGGTGA CTCCGTC
3-ACP0pET30LIC	GAGGAGAAGCCCGGTTACGGTGCCGAGGCGCCCGG
5-ACP1pET30LIC	GACGACGACAAGATGGCGGTGCCGGCCGACGAA
3-ACP1pET30LIC	GAGGAGAAGCCCGGTTACGGAACACGCTCTGCCGC
5-ACP2pET30LIC	GACGACGACAAGATGCGGGTCCGCTCCACCGAG
3-ACP2pET30LIC	GAGGAGAAGCCCGGTTAGGTGGTGGCCGGGGTGTC
5-ACP3pET30LIC	GACGACGACAAGATGACCGCCGCGGCACGAGCC
3-ACP3pET30LIC	GAGGAGAAGCCCGGTTAGGCTTCCGTCGCCTCCGG
5-ACP4pET30LIC	GACGACGACAAGATGGGCGTGCCGACGCCCGAC
3-ACP4pET30LIC	GAGGAGAAGCCCGGTTAGACGGCCGGAGCGGTGGA
5-ACP5pET30LIC	GACGACGACAAGATGTGGCCCGAGCCCGCAGCG
3-ACP5pET30LIC	GAGGAGAAGCCCGGTTACGGCTCGGGTGTGGCGGT
5-ACP7pET30LIC	GACGACGACAAGATGCGCGCCCTGCTCGACGCG
3-ACP7pET30LIC	GAGGAGAAGCCCGGTTACACCGGAGCCGGTGCCGG
5-ACP8pET30LIC	GACGACGACAAGATGACCGCACCCACGGCGCAGCCG
3-ACP8pET30LIC	GAGGAGAAGCCCGGTTACTGGGCCGGCGCGGGCCG
5-ACP9pET30LIC	GACGACGACAAGATGTGGATCCGTTCCGCGGCC
3-ACP9pET30LIC	GAGGAGAAGCCCGGTTAGTCGTCCGTTCCGGCTCCGC
5-ACP10pET30LIC	GACGACGACAAGATGCGCGACGACGGCGTACCG
3-ACP10pET30LIC	GAGGAGAAGCCCGGTTAGGGCTCGGTCCGCTTGCCACC
5-ACP11pET30LIC	GACGACGACAAGATGACCACCGATCCCGGGACT
3-ACP11pET30LIC	GAGGAGAAGCCCGGTTACTGCGGCGCGGCACCGGCTC
5-ACP12pET30LIC	GACGACGACAAGATGCGGATCCGGGACGCCGCG
3-ACP12pET30LIC	GAGGAGAAGCCCGGTTACTCCGTACGGCGGTCCGCCG
5-ACP13pET30LIC	GACGACGACAAGATGCCCCCGTACTGCGCGGC
3-ACP13pET30LIC	GAGGAGAAGCCCGGTTAGTCGAAGTCGGCGAACAG
5-ACP14pET30LIC	GACGACGACAAGATGCCGGCCCGGCGTACGGCC
3-ACP14pET30LIC	GAGGAGAAGCCCGGTTAGTCGTCCGCGGTGCCGAC
5-ACP15pET30LIC	GACGACGACAAGATGCCGGGCCACGCCACCGGTTCC
3-ACP15pET30LIC	GAGGAGAAGCCCGGTTATTCCCCTCGCTCATTCGCGT

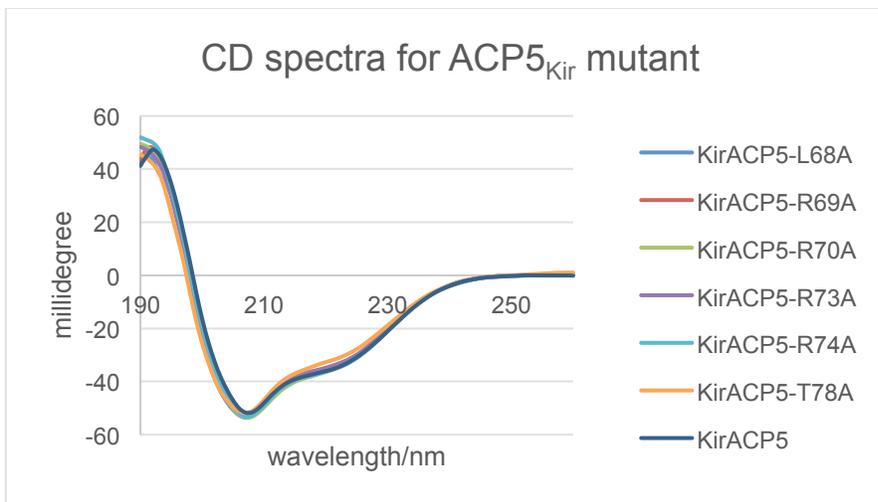
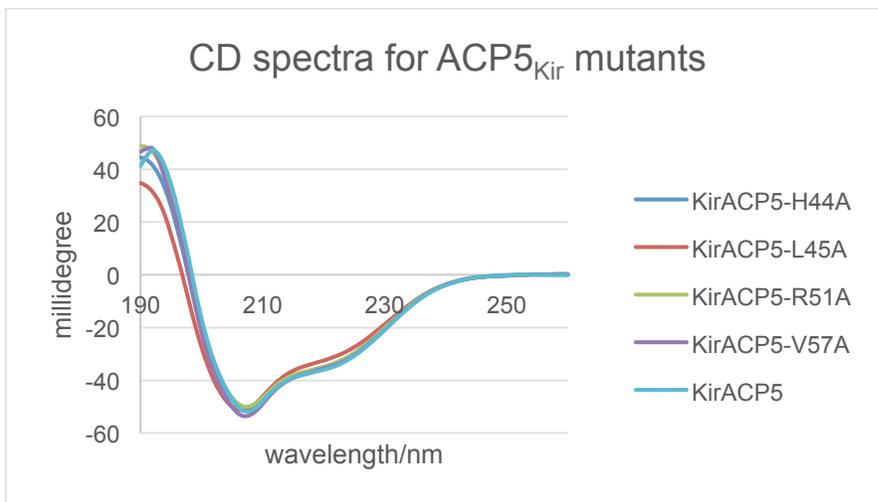
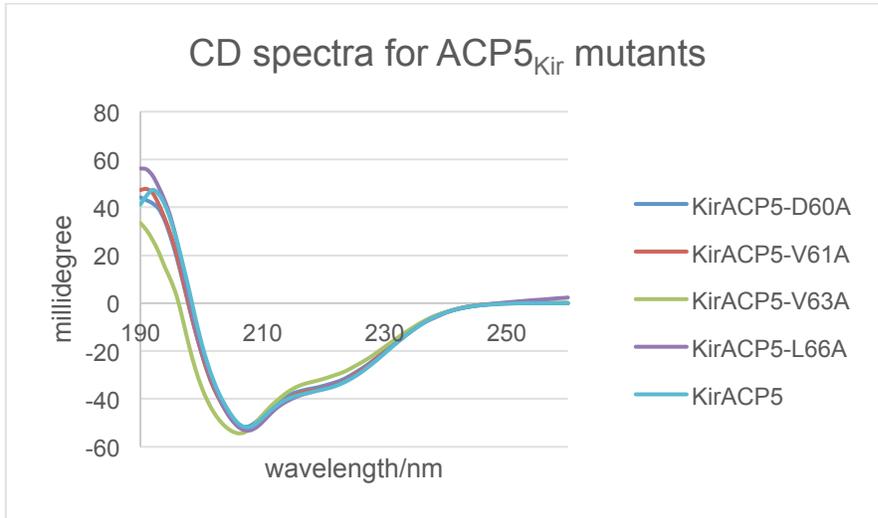
**Table S3:** Oligonucleotides used for construction of ACP10<sub>Kir</sub>/ACP5<sub>Kir</sub> chimeras, related to 'Site-Directed Mutagenesis and Chimeragenesis of ACP's and KirCII' and Figure 7.

ACP10 <sub>Kir</sub> NA5-1F	PCR 1	AGCTTCGGTACCGATGACGACGACAAGATGTGG
ACP10 <sub>Kir</sub> NA5-1R	PCR 1	GGCCAGGTTCTGCAGGCCAGCGAGTCCACACCGACGTC
ACP10 <sub>Kir</sub> NA5-2F	PCR 2	GACGTCCGGTGTGGACTCGCTGGGCCTGCAGAACCTGGCC
ACP10 <sub>Kir</sub> NA5-2R	PCR 2	AGCTTCCCATGGTTGAGGAGAAGCCCGGTTAG
ACP10 <sub>Kir</sub> CA5-1F	PCR 1	AGCTTCGGTACCGATGACGACGACAAGATGCG
ACP10 <sub>Kir</sub> CA5-1R	PCR 1	GGCGGCGCAGCCCGAAGGAGTCCAGGCCGTACTION
ACP10 <sub>Kir</sub> CA5-2F	PCR 2	CGAGTACGGCCTGGACTCCTTCGGGCTGCGCCGCC
ACP10 <sub>Kir</sub> CA5-2R	PCR 2	AGCTTCCCATGGTTGAGGAGAAGCCCGGTTAG
ACP10 <sub>Kir</sub> L0A5-1F	PCR 1	AGCTTCGGTACCGATGACGACGACAAGATGTGG
ACP10 <sub>Kir</sub> L0A5-1R	PCR 1	GCAGCTGGGTGGGGAGTTCTGACTCCGGGTGCGCTCC
ACP10 <sub>Kir</sub> L0A5-2F	PCR 2	GGAGGCGACCCGGAGTCAGAACTCCCCACCCAGCTGC
ACP10 <sub>Kir</sub> L0A5-2R	PCR 2	AGCTTCCCATGGTTGAGGAGAAGCCCGGTTAG
ACP10 <sub>Kir</sub> HIA5-1F	PCR 1	AGCTTCGGTACCGATGACGACGACAAGATGTGG
ACP10 <sub>Kir</sub> HIA5-1R	PCR 1	CGGACGGGGCGATCCCCAGGTGCGCGGCGAGC
ACP10 <sub>Kir</sub> HIA5-2F	PCR 2	GCTCGCCGCGCACCTGGGGATCGCCCCGTCCG
ACP10 <sub>Kir</sub> HIA5-2R	PCR 2	AGCTTCCCATGGTTGAGGAGAAGCCCGGTTAG
ACP10 <sub>Kir</sub> LIA5-1F	PCR 1	AGCTTCGGTACCGATGACGACGACAAGATGCG
ACP10 <sub>Kir</sub> LIA5-1R	PCR 1	CGGTCCGGTGCCATGCCGAGGATCCCGCCGATCAGCTC
ACP10 <sub>Kir</sub> LIA5-2F	PCR 2	GACGTCCGGTGTGGACTCGCTGGGCCTGCAGAACCTGGCC
ACP10 <sub>Kir</sub> LIA5-2R	PCR 2	AGCTTCCCATGGTTGAGGAGAAGCCCGGTTAG
ACP10 <sub>Kir</sub> LIA5-3F	PCR 3	GAGCTGATCGGCGGGATCCTCGGCATGGCACCCGGACCG
ACP10 <sub>Kir</sub> LIA5-3R	PCR 3	GGCCAGGTTCTGCAGGCCAGCGAGTCCACACCGACGTC

**Figure S1:** LC-MS analysis of the conversion of apo-ACP5<sub>Kir</sub> to holo-ACP5<sub>Kir</sub>. These spectra show the complete Sfp-catalyzed phosphopantetheinylation of wild-type apo-ACP5<sub>Kir</sub> as a representative example, related to 'Cloning and Expression of Kirromycin ACP's'.



**Figure S2:** CD spectra of wild-type and mutant ACP5<sub>Kir</sub>. These spectra show that the mutations analyzed do not significantly alter the secondary structure, compared to the wild-type ACP, related to 'Cloning and Expression of Kirromycin ACP's'.





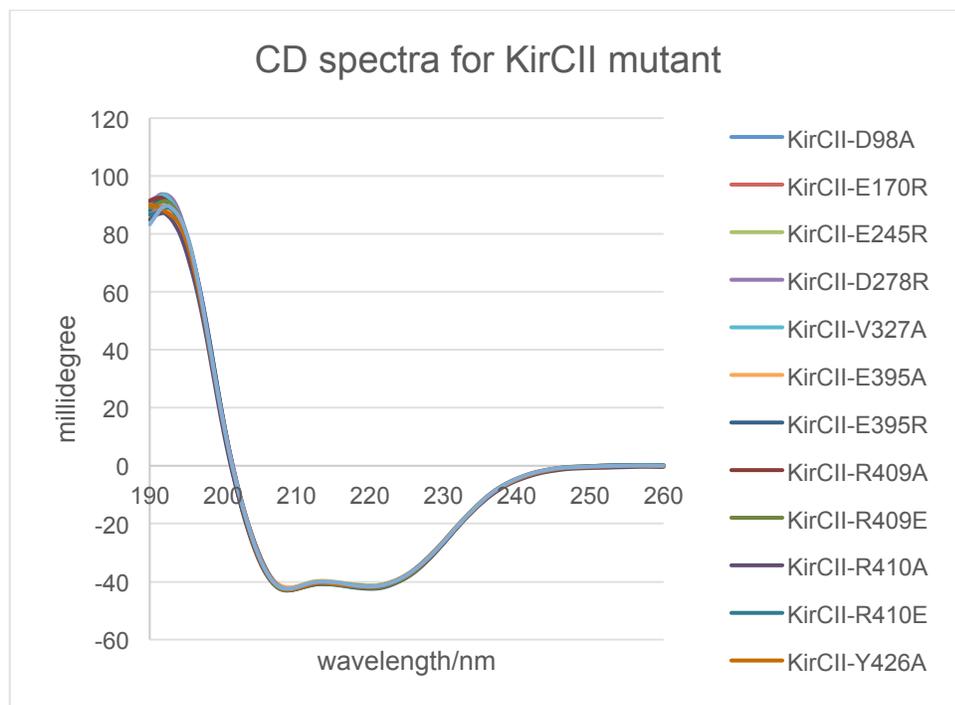
KirCII CRMVRC TVASHSHYVDELRDDLAGALRP-LSPVPSRVPPFYSTVTAAPVPGTDLGPAYWMR 333  
 AT1 AKRLAVDYASHSSHVETIRDALHAELGEDFHPLPGFVPPFSTVTGRWTQPDELDAGYWYR 348  
 AT2 ARAIPVDYASHSTAHPVPRDELVQALAG-ITPRRAEVPPFSTLTGDFLDG TELDAGYWYR 348  
 AT3 VRDIDVDYASHSPQIERVREELLETTGD-IAPRPARVTFHSTVESRSMDG TELDARYWYR 345  
 AT4 PRRIAVRYASHSPEVARIEDRLAAELGT-ITAVRG SVPLHSTVTGEVIDTSAMDASYWYR 342  
 AT5 ARRIPVDYASHSPQVESLREELLTELAG-ISPVSADVALYSTTTGQPIDTATMDTAYWYA 338  
 AT6 AKTLPVDYASHSRHVEEIRETILADLDG-ISARRAAIPLYSTLHGERRDGDADMGPYRYWD 345  
 : : \*\*\*: : : : : : . . . : : .\*\* . : : \*\*

KirCII NLREPVR LAAATGR LAEDGHEIFVEVSTHPVLLSSLRQTLESAGRPGEVLP SGRRRTERR 393  
 AT1 NLRRTVRFADAVRALAEQGYRTFLEVSAHPILTAAIBEEIGDGS GA----- 393  
 AT2 NLRHPVEFHS AVQALTDQGYATFIEVSPHPVLASSVQETLDDA----- 391  
 AT3 NLRET VRFADAVTR LAESGYDAFIEVSPHPVVVQAVEEAVEEADG----- 390  
 AT4 NLRRPVLF EQAVRGLVEQGFDTFVEVSPHPVLLMAVEETA EHAGA----- 387  
 AT5 NLREQVRFQDATRQLAEAGFD AFVEVSPHPVLTVG IEATLDSALP----- 383  
 AT6 NLRSQVRFDEAVSAAVADGHATFVEMSPHPVLTAAVQEIA----- 385  
 \*\*\* \* : \* . \* \* : \* : \* : : . .

KirCII AMLSSLGLTFTYGRDPHWPTSAPPAPALTPYQAAVLAARRRPRPSPAAAGSG 445  
 AT1 -----DLS---AIHSLRRGDG----- 406  
 AT2 -----ESDAAVLGLTERDAGD----- 407  
 AT3 -----AED-AVVVGLSLHRDGGD----- 406  
 AT4 -----EVT---CVPTLRRE----- 398  
 AT5 -----ADAGACVVGTLRRDRG----- 399  
 AT6 -----ADAVAIGSLHRDTAE----- 400  
 : : : \*



**Figure S5:** CD spectra of wild-type and mutant KirCII, related to Figure 6.



**Figure S6:** Secondary structure prediction of ACP5<sub>Kir</sub> using PSIPRED, related to Figure 7. The predicted secondary structure includes coiled regions (L<sub>0</sub> and L<sub>III</sub>) that flank the N- and C-terminal helices.

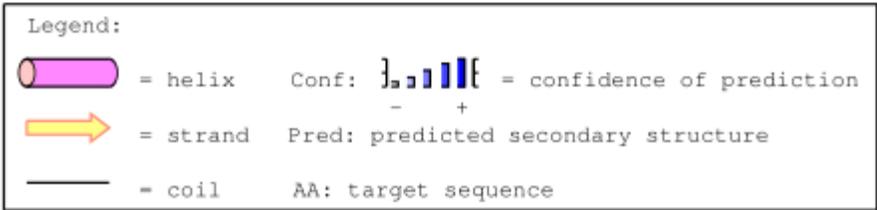
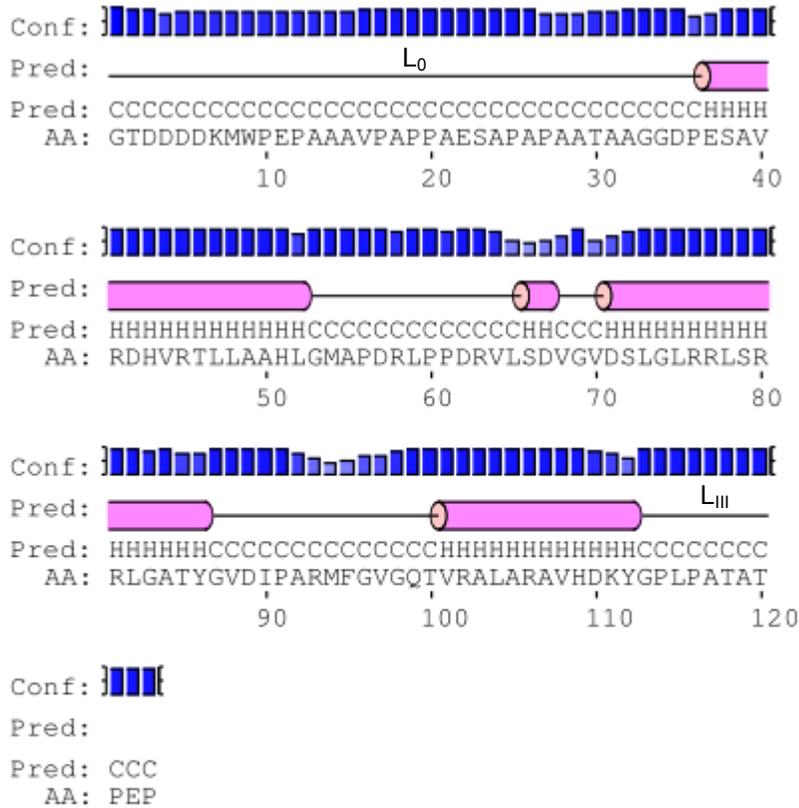


Figure S7: Amino acid alignment of various ACP's, related to Figure 7.

